Malaysian Journal of Analytical Sciences, Vol. 7, No. 2 (2001) 323-326

Environmental Biomonitoring: The Possibility of Using Preserved Biological Specimen

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(Received 6 September 2000)

Abstract: Environmental monitoring has become a very important tool in environmental management. However, it is very frequent that some important data on the initial status or conditions of a particular area is not available. This obstructs efforts to rehabilitate a disturbed area or to determine the changes that have taken place over a specific period of development. Biological sampling has been widely practiced by biologists over centuries and it is more likely to find preserved biological specimen of a particular area in museums and laboratories. This paper is to explore the possibility of using the preserved biological specimen to determine/estimate the historical environmental status of the area. The research was conducted using freshwater molluscs collected from Bau District in Sarawak. The samples collected at each site were divided into two portions, one would be analysed for heavy metals immediately while the other portion was preserved in methanol for 6 months before the chemical analysis. The results showed that the metals contents in the tissue of the molluscs differ significantly between the fresh and preserved specimen. However, the contents of metals in the shell of the animals did not indicate any significant variation. The variation of metal contents in the tissues may be related to the solubilisation of fatty tissues by methanol thus causing the metal content to increase per unit weight of the dried tissue. This paper concludes that there is a possibility of using preserved samples for biomonitoring of the earlier environment, however much work on the validation and this approach need to be done.

Abstrak: Pemantauan alam sekitar kini merupakan aktiviti pengurusan alam sekitar yang penting. Seringkali keadaan asal sesuatu kawasan yang ingin dikaji tidak dapat diperolehi. Kekurangan pengetahuan keadaan asal sesuatu tempat merumitkan usahan pemulihan kawasan tersebut. Pengumpulan sample-sampel biologi telah lama dilakukan dan ini mungkin menjadi satu punca data yang boleh diterokai. Kertas kerja ini bertujuan meneroka kesesuaian penggunaan sample-sampel biologi terawit bagi menentukan keadaan semulajadi sesuatu kawasan. Kajian dijalankan mengguna sample siput dari Bau, Sarawak. Sampel-sampel siput dikutip dan dibahagi kepada dua bahagian, satunya dianalisis kandungan logam berat dengan serta merta dan satu lagi bahagian diawit di dalam methanol untuk 6 bulan sebelum dianalisis. Keputusan kajian menunjukkan terdapat perbezaan ketara di antara kandungan logam berat di dalam tisu siput yang diawit dan yang segar. Tiada perbezaan signifikan di antara cengkeran yang diawit dan yang segar. Perubahan kandungan logan berat di dalam tisu berkemungkinan disebabkan oleh penglarutan tisu-tisu lemak kedalam methanol dan ini menghasilkan peningkatan kandungan logam berat di dalam tisu siput yang diawit. Kajian ini membuat kesimpulan bahawa penggunaan sampel biologi terawit dalam pemantauan alam sekitar adalah menggalakan. Mamun demikian kajian lanjutan harus diteruskan bagi menjamin kesahihan teknik ini.

Keywords: preserved biological specimen, environmental monitoring, molluscs heavy metals.

Introduction

Preserving and conserving the environment of the earth has become an issue that attracts worldwide attention. The world population has finally become more aware of the importance of sustaining our present environment to the continual survival of human civilization. Several world summits have been held to discuss the concerted effort that each nation in the world can undertake to sustain our world environment if improving it is not possible. Out of these summits, several declarations have been made such as the Montreal Protocols (1987), Langkawi Declaration (1989) and the resolutions made at the Earth Summit at Rio de Janeiro (1992). All these declarations propose approaches and steps to be taken to conserve our earth. The successful implementations of these proposed actions are vital and therefore continuous effort in monitoring the states of the environment is crucial. The objectives of the monitoring exercise are to determine if the actions taken are sufficient or correct and what further actions can we take to improve the situations.

Among the many environmental monitoring approaches, monitoring of biological species has received much attention from many environmental scientists. The reason for such an interest in biomonitoring is obvious, as the well being of the ecosystem will reflect the overall health of our environment. Many flora and fauna species, of both terrestrial and aquatic, have been selected as biomonitors for various pollutants and among them is the heavy metals in the environment particularly in surface water. Hundreds of articles have been published on this subject, [1] & [2], and the Zebra Mussels Watch Project has been the most widely documented [3]. The procedures in using aquatic fauna, as biomonitors for heavy metals have been well-established and analytical procedures are reliable and well documented. The advantages of the biomonitoring have been discussed at great length in many other authors, [4]&[5]. However, the practice of biomonitoring is limited to a few and only in recent time that this approach has been widely used. Many environmental sensitive location has not been

monitored and the chances of collecting samples in those area is no longer possible as most of the biota there would have been killed due to extreme environmental conditions. Some areas are rather fortunate that biological samples, such as molluscs, fishes, frogs and plants have been preserved by biologists. It would be of great value if such preserved samples could still be used for monitoring purposes.

This paper is aimed at establishing the possibility of using preserved samples for monitoring of the past environmental conditions. It is essential for us to establish the validity of the preserved samples in terms of the retention of pollutants by the specimen over time in the various preservatives. The study was to determine if there is variation of heavy metals concentrations between the fresh molluscs specimen and those preserved in methanol for a period of six months.

Methodology

Sample Collection and Preservation.

The sampling sites for this work were located in the upper catchment of Sg. Sarawak. There were a total of six sampling locations and at each site, mollusc samples (*Brotia costula*) were collected. The molluscs collected at each site were of similar size (cone length of ca. 30 mm) and a minimum of 20 individuals were selected. Each batch of samples was divided equally into two groups. One group of molluscs was processed immediately for metal analysis while the other group was preserved in a specimen jar and methanol was added to fully immerse the specimen. The methanol soaked specimens were kept on shelf for 6 months at room temperature (25^{o}C) before analysis on their metal contents were conducted.

Sample Preparation.

Fresh molluscs specimens were cleaned by rinsing with tap water and the tissues were separated from its shell manually using forceps. It was found that the separation was easier if the molluscs were first killed by soaking them in hot water for about 1 – 2 min. The separated whole soft tissues and shells were oven dried at 60^{o}C and later crushed to fine powder using a pastel and mortar. The crushed samples were kept in desiccators for future analyses.

The methanol preserved specimens were prepared in a similar way. The samples were removed from the preservation solution and the tissue was separated from its shell. The tissues and shells were washed with water separately and dried in oven at 60^{o}C. The dried samples were ground to powder and stored in desiccators.

0.5 g of the crushed sample was digested with 5 mL of conc. HNO_3 and 2 mL of 30 % v/v H_2O_2 in a microwave digester (Milestone Mega 1200), [6]. The digested samples were diluted to 50 mL with distilled water in volumetric flasks. These solutions were analyzed for metals (Cd, Co, Cu, Ni and Pb) using the atomic absorption spectrometer, Perkin Elmer Model 3110 AAS.

Results and Discussion

The metal contents in freshly collected mollusc samples and their preserved counterparts were analyzed and recorded for their various locations. Table 1 shows the results of these analyses.

The analyses have shown that all the preserved samples have significantly ($p = 0.05$) higher metal contents as compared to their fresh samples. The differences were more obvious for the tissues than for the shell, with the preserved tissues showed increases between 44% and 98 % with the exception for Ni where the increase was almost 250%. The increase in the Cr, Cu and Pb contents in the preserved tissues were comparable to each other (44 – 68%) while that of Cd was moderately higher at 98% increase. The increase in metal contents of the preserved shells were in the range of 10 – 63% with an exception to Ni where the increased was recorded at over 200%. Table 2 shows the percent increased in metal contents of the preserved samples over the fresh samples. The percentage was calculated according to the following equation:

$$\%Increase = \frac{[Metal]_{preserved} - [Metal]_{fresh}}{[Metal]_{fresh}} \times 100 \qquad \text{(Eq. 1)}$$

Increases in the metal contents for the preserved tissues may be attributed to the dissolution of the fatty tissues of the molluscs by the preservation solution (methanol) and the dehydration of the samples by methanol. The removal of fatty tissues is believed to of greater significant as the animals usually contain about 30% fat. A dry weight loss of the mollusc tissues soak in ethanol has been recorded to be more than 60%, [7]. This proportional weight loss would be expected in the case of our study. However, quantitative measurement on the weight loss of the molluscs tissues soak in methanol was not studied in this work. If metals are uniformly distributed in the tissues, the loss of such fatty tissues may not directly responsible for the drastic increase in the overall metal content, as the metal would also be dissolved in the similar process. Therefore this significant increase in metal content in tissue suggests strongly that metals are distributed unevenly and more often that not they are organ specific.

The concentration of different metal at different organs or muscles also may attribute to the different percentage of metal variations in the samples. For example, Cu and Pb were rather similar with 44 % increased in the preserved sample while Cr was 68%, Cd was 98% and Ni was 248% more. This suggests that Ni embedded in the tissues that is highly insoluble in the preservation solution while Cu and Pb are attached to the more readily soluble tissues.

Table 1: The metal contents in the tissues and shells of the freshly collected molluscs and its methanol-preserved counterparts.

Site	Cd (mg/kg)		Cr (mg/kg)		Cu (mg/kg)		Ni (mg/kg)		Pb (mg/kg)	
	fresh	presvd	fresh	presvd	fresh	presvd	fresh	presvd	fresh	presvd
Tissue										
A	1.99 ± 0.01	4.32 ± 0.57	4.97 ± 0.02	6.98 ± 0.01	52.33 ± 1.43	76.14 ± 0.48	3.64 ± 0.56	15.63 ± 0.58	33.45 ± 1.16	32.92 ± 2.67
B	1.98 ± 0.01	2.32 ± 0.59	3.63 ± 0.59	7.94 ± 0.25	59.01 ± 6.17	102.93 ± 1.72	5.27 ± 0.58	20.51 ± 4.66	16.47 ± 1.43	21.51 ± 1.14
C	1.98 ± 0.01	3.65 ± 0.58	3.96 ± 0.01	8.29 ± 1.14	57.73 ± 0.56	85.92 ± 6.06	3.30 ± 0.56	12.94 ± 0.98	27.71 ± 2.55	31.85 ± 1.73
D	1.98 ± 0.01	2.96 ± 0.01	4.63 ± 0.56	8.56 ± 0.86	55.16 ± 1.45	63.17 ± 0.82	1.98 ± 0.01	13.17 ± 2.88	27.10 ± 2.57	25.66 ± 0.08
E	1.00 ± 0.01	2.97 ± 0.02	4.98 ± 0.05	8.97 ± 1.01	56.78 ± 2.60	98.70 ± 0.78	12.62 ± 1.51	13.86 ± 0.08	9.96 ± 0.99	23.44 ± 0.57
F	1.33 ± 0.57	2.97 ± 0.01	8.64 ± 0.58	6.59 ± 0.56	40.87 ± 3.54	43.51 ± 0.91	8.64 ± 0.57	8.90 ± 0.02	15.62 ± 3.76	32.63 ± 4.30
Shell										
A	2.97 ± 0.02	3.31 ± 0.58	6.93 ± 0.05	8.27 ± 0.57	19.14 ± 0.42	24.14 ± 0.55	4.95 ± 0.04	28.11 ± 2.09	35.05 ± 1.49	47.19 ± 1.70
B	2.97 ± 0.01	3.64 ± 0.58	7.29 ± 0.04	10.25 ± 0.55	19.48 ± 0.64	23.82 ± 1.07	6.27 ± 0.55	26.13 ± 2.24	19.14 ±1.43	38.04 ± 2.17
C	2.99 ± 0.01	2.96 ± 0.02	5.98 ± 0.01	10.53 ± 0.60	21.60 ± 4.73	19.03 ± 0.71	4.98 ± 0.01	15.13 ± 1.04	26.25 ± 1.21	37.84 ± 0.84
D	2.98 ± 0.01	3.30 ± 0.58	5.95 ± 0.03	11.55 ± 0.65	23.48 ± 0.48	19.14 ± 0.68	3.97 ± 0.02	13.53 ± 0.63	23.15 ± 0.67	34.64 ± 0.75
E	1.32 ± 0.57	3.30 ± 0.56	12.25 ± 0.55	14.86 ± 1.11	10.60 ± 0.58	18.48 ± 0.63	20.14 ± 2.64	32.11 ± 2.97	22.52 ± 0.53	38.28 ± 3.70
F	1.99 ± 0.01	2.98 ± 0.01	11.27 ± 1.49	15.21 ± 0.52	10.91 ± 0.98	16.57 ± 0.61	11.60 ± 0.55	14.55 ± 0.54	17.57 ± 2.04	41.98 ± 2.77

In the case of the shells of the gastropods, it is surprising to notice a similar increase as the case of the tissues in the metal contents. One would not expect much dissolution of the shell by methanol. The most probable explanation for such increase is the removal of the membrane layer that lined the shell from the inside by the solvent. In the fresh sample this membrane was strongly attached to the inner layer of the shell and they were not removed. In the preserved samples this lining membrane may have been removed and hence there was a slight loss in weight. The loss of weight due to the membrane may not be enough to substantiate the amount of metal increase and there may be other elution or deposition kinetics involved. One possible pathway for the increase would be the dissolved metals, from the tissues, in the methanol may be deposited or adsorbed onto the shell. The abnormality that was observed was for the two folds increase in Ni content. No explanation could be offered for this observation at this stage.

The possibilities of using preserved samples for determining the levels of metals in the past environment may still be feasible despite our finding that there seems to be an increase in the metal contents. The usability of the preserved samples, at this stage should only be limited to obtaining estimation for environmental planning an evaluation purposes. The absolute values obtained from the preserved samples cannot be used, as these measurements are not at all accurate. More method development work must be conducted before any meaningful conclusion to the feasibility of using preserved samples can be made. Other important factors need to be established are the influence of the duration of he preservation, the type of preservative used, and the type of specimen preserved.

Table 2: Average increases of metal contents in the preserved sample over the fresh samples.(Calculations are based on Eq. 1)

	Cd	Cr	Cu	Ni	Pb
Tissue	98%	68%	44%	248%	44%
Shell	40%	48%	10%	202%	63%

Conclusion

The used of preserved biological specimen for monitoring of heavy metals in the aquatic environment has provide early indication of its usefulness. However for more precise and accurate quantitative measurement, further detailed method development is required. This will hopefully standardize a common procedure and more comparable and consistent results would be obtained.

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